Field Crops Research 217 (2018) 172–179

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Forage rape (*Brassica napus* L) seed quality: Impact of heat stress in the field during seed development

Muhammad Rashid[a,e], John G. Hampton[a,*], M. Phil Rolston[b], Jason A.K. Trethewey[c], David J. Saville[d]

[a] Seed Research Centre, Lincoln University, Lincoln 7647, New Zealand
[b] Foundation for Arable Research, Templeton, Christchurch, 8445, New Zealand
[c] Syngenta Crop Protection Ltd, Auckland 1010, New Zealand
[d] Saville Statistical Consulting, Lincoln 7640, New Zealand
[e] Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS), Uthal 9015, Pakistan

ARTICLE INFO

Keywords:
Heat stress
Hourly thermal time
Forage rape
Seed quality
Temperature

ABSTRACT

Climate change is predicted to increase the frequency of heat stress events during seed production. In two consecutive seasons, plants within a forage rape (*Brassica napus* L.) seed crop were covered with plastic sheeting to increase ambient air temperature for the period between seed filling (80% seed moisture content) and seed physiological maturity (50% seed moisture content) = T_1, between physiological maturity and harvest (14% seed moisture content) = T_2, and between 80% seed moisture content and harvest = T_3. This resulted in 47 and 102 h when air temperature exceeded 25 °C for T_1, 121 and 173 h for T_2, and 145 and 228 h for T_3 compared with 9 and 30 h for the uncovered control in each season respectively. Hourly thermal time (T base = 25 °C) was calculated for each treatment. Both T_1 and T_2 resulted in small (2–10%) reductions in germination in each season, but when season data were meaned and analysed, only T_2 and T_3 significantly reduced germination. Seed mass (as measured by thousand seed weight) was significantly reduced by T_1, but not T_2, while seed vigour, as assessed by the accelerated ageing and conductivity tests, was significantly reduced by all three treatments, with $T_3 > T_2 > T_1$. The number of hours that temperature exceeded 25 °C was negatively correlated with germination and seed vigour, but not seed mass. Approximately 100 h of temperature exceeding 25 °C, or an hourly thermal time of 300 °C h (T_b = 25 °C) were required to reduce the vigour status of the seed lot. Crop management strategies to avoid heat stress during seed development are unlikely to succeed in this environment.

1. Introduction

Predicted increased ambient temperature variability can result in short durations of heat stress, which if coinciding with plant reproductive processes, can reduce seed yield (Prasad et al., 2017) and seed quality (Rashid et al., 2017a). Prasad et al. (2017) reported that day-time temperatures during the reproductive phase which caused significant damage to reproductive processes ranged between 30° to 40 °C depending on species.

High quality seed is a crucial input for successful crop production, particularly because of the increasing uncertainty due to climate change (Finch-Savage and Bassel, 2016). Heat stress during seed development can reduce seed mass, germination and seed vigour (Prasad et al., 2008; Hampton et al., 2013; Singh et al., 2013). Seed vigour loss has been associated with heat stress both before (Spears et al., 1997) and after (Shinohara et al., 2006a,b) seeds reach physiological maturity.

In the Canterbury Province of New Zealand, where over 90% of the country's seed crops are produced (Hampton et al., 2012), the climate is described as cool temperate, and daily mean ambient temperatures do not usually exceed 25 °C, even in summer (Shinohara et al., 2006a). However, maximum temperatures in Canterbury can exceed 30 °C during the reproductive growth of seed crops (Rashid, 2016), and Shinohara et al. (2006a) reported a total of up to 21 h above 30 °C during early seed development (70–80% seed moisture content), and up to 45 h above 25 °C during the same period.

Shinohara et al. (2006b) reported that exposure of garden pea (*Pisum sativum* L.) plants to a short heat stress period of 4 days at 30 °C day/25 °C night (240 °C h) both before and after seeds had reached physiological maturity had no effect on the germination of the harvested seed but significantly reduced seed vigour. More recently Rashid et al. (2017a) applied the same heat stress to forage rape (*Brassica napus* L.) plants both before and at seed physiological maturity, and also

* Corresponding author at: Seed Research Centre, PO Box 85084, Lincoln University, Lincoln 7647, New Zealand.
E-mail address: john.hampton@lincoln.ac.nz (J.G. Hampton).

https://doi.org/10.1016/j.fcr.2017.10.022
Received 27 July 2017; Received in revised form 24 October 2017; Accepted 31 October 2017
Available online 04 January 2018
0378-4290/

recorded a signficant reduction in seed vigour. Both these studies were conducted in a controlled environment, and information about the effects of heat stress at specific times during seed development from field studies is lacking (Aksouh-Harradj et al., 2006). In New Zealand, climate change is predicted to increase mean ambient air temperature and increase the frequency of heat stress events (NIWA, 2012). The current study was initiated to determine the effect of elevating day and night temperature on seed quality in a field environment.

2. Materials and methods

2.1. Field experiments

The first field trial was conducted in the 2011–12 season. Plots of forage rape (cv. Greenland) were sown on 25 March 2011 at the AgResearch Farm, Lincoln, Canterbury (43 °C 38'S, 172° 28'E) at 3 kg seed ha^{-1} using a cone-seeder with sowing depth of 2 cm. Plot size was 10 m x 2.7 m and row spacing was 15 cm. Crop management was as described by Trethewey (2012). The plots used for the current experiment were the control (untreated) plots of Trethewey (2012). The second field trial was conducted in the 2012-13 season, using a different field at the AgResearch Farm. The only difference in management from the previous season was the sowing date of 21 March 2012.

From the onset of pod development (10–15 days after peak flowering), seed moisture content was monitored to assess seed development stages. Pods from three randomly selected plants were collected from the middle section of the raceme and the seeds removed by hand threshing. Seed moisture content (SMC) was immediately determined using the low constant temperature oven method (ISTA, 2016). These assessments continued weekly until seed harvest.

Ambient air temperature was artificially increased by covering a 2.7 × 2 m section of each plot with plastic sheeting (60 µm low density clear polyethylene; Egmont Commercial Ltd, Christchurch) around all four sides and over the top (Fig. 1.). As can be seen in Fig. 1, the area was not completely enclosed, so that there was still free air movement. There were four treatments, each replicated four times *viz* plot covered from seed filling (≃80% SMC) to physiological maturity (50% SMC) = T_1, plot covered from physiological maturity to seed harvest (14% SMC) = T_2, plot covered from 80% SMC to seed harvest = T_3, and an uncovered control. Once seeds had reached physiological maturity all plots were covered with netting to prevent bird damage to seeds.

2.2. Environmental data

Air temperature and relative humidity were recorded hourly for all treatments using HOBO series-8 data loggers (MicroDAQ, Com. Ltd, USA). The data logger in each plot was supported on a wooden pole approximately 75 cm above the plant canopy with the temperature sensor parallel to the ground surface. Data were downloaded weekly. Hourly mean temperature data were converted to the number of degree hours exceeding a base temperature (Tb) of 25 °C (for a minimum of 1 °C increase above 25 °C, not less than 1 °C). Hourly thermal time was also calculated as an integral of hourly temperature above Tb by using the formula ΣTmin + Tmax/2-Tb], where Tb, Tmin and Tmax are the base, minimum and maximum temperatures, respectively.

2.3. Seed harvest and seed quality

At harvest (14% SMC) all plants from within each plot (but excluding border rows) were cut at the base of the raceme. All pods were then removed from the plants by hand and seeds removed from the pods by hand. Harvested seeds were then tested for quality by determining seed germination, seed vigour (using the accelerated ageing and conductivity tests) and seed mass (thousand seed weight) using internationally accepted methodology (ISTA, 2016).

2.4. Statistical analysis

The four treatments in each year were arranged in a randomized complete block design, with four replications of each treatment. The effect of heat stress (T_1 and T_2) on seed quality was analysed as treatment factors. Each treatment factor had two levels, T_1 (+, −) and T_2 (+, −), which made up a 2 × 2 factorial design.

Results for the seed quality tests were analysed using an analysis of

Fig. 1. A section (2.7 × 2 m) in each forage rape field plot covered with plastic sheeting to artificially elevate temperature.

variance for four blocks with 2 × 2 factorial treatments. Quality test means were compared using the least significant difference (LSD) at P = 0.05 using Genstat Software (16th Edition, VSN International Ltd, Hemel Hempstead, UK). The interaction between the two treatments was calculated, and least significant interaction (LS interaction (5%) = LSD × √2) was used to test for interaction significance. A combined analysis of two years data was conducted to estimate the magnitude of treatment effects over the two seasons by inputting the treatment means for each season into an analysis of variance that had a blocking factor of "season" and treatments the 2 × 2 factorial structure.

Correlations between (*x*) hourly thermal time and (*y*) seed quality (germination, germination after accelerated ageing, conductivity and thousand seed weight), and (*x*) the 'number of hours when temperature exceeded 25 °C' and (*y*) seed quality were determined using analysis of covariance (ANCOVA). For each of the eight ANCOVAs, the four treatment means for each season and variable were input, and season was treated as a 'treatment'; this enabled the correlations to be pooled between seasons.

3. Results

3.1. Environmental conditions

Daily maximum and minimum field temperature during phase-I of seed development, from 80% SMC to physiological maturity, was 27.2° C and 6.9° C in 2011–12 (Fig. 2) and was 29.3° C and 3.4° C in 2012–13 (Fig. 3). During phase-II, from physiological maturity to 14% SMC, these temperatures were 28.4° C and 3.4° C in the first season (Fig. 2) but in the second season were 31.6° C and 2.5° C (Fig. 3).

Ambient air temperature was warmer in the second season than in the first season, with 30 h above 25° C being reached over this time in 2012-13 compared with only 9 h in 2011–12. Hourly thermal time above the T_b of 25° C was 15.6° C h in the first season and 30.0° C h in the second season (Table 1). The difference was mostly explained by the warmer temperature both before and after physiological maturity in the 2012-13 season.

T_1 increased the time to reach physiological maturity by 3-4 days. This delay was mostly explained by slower seed moisture loss due to increased humidity inside the covered plots (Table 1). During this time there were 40 h when the temperature exceeded 25 C° in the first season and 84 h in the second season, resulting in an hourly thermal time of around 101.3° C h and 255.5° C h, respectively. T_1 increased hourly thermal time by 111° C h and 283° C h, respectively, while the number of hours > 25° C after the covers were removed, were 47 and 102 h for the 2011–12 and 2012-13 seasons, respectively. Likewise, T_2 increased hours above 25° C by 118 h in 2011–12 and 159 h in 2012-13, which resulted in an hourly thermal time of 334.4° C h and 577.6° C h in the 2011–12 and 2012-13 seasons, respectively (Table 1). Therefore, the total time above 25° C and hourly thermal time for T_2 was 121 h and 340.1 ° C h in the 2011–12 season and 173 h and 605.7° C h in the 2012-13 season (Table 1).

For T_3 there were 149 h above 25° C and hourly thermal time was 425.2° C h in the 2011–12 season. During the 2012-13 season, there were 228 h above 25° C which increased hourly thermal time to 783.2° C h (Table 1).

3.2. Seed quality

A negative influence of elevated temperature on seed germination was recorded in both seasons (Table 2). Heat stress treatment both before (T_1) and after (T_2) physiological maturity reduced germination. T_1 significantly reduced germination in both seasons ($P < 0.05$ and $P < 0.001$, respectively) but T_2 had a greater effect than T_1 on reducing seed germination in both seasons ($P < 0.001$). However, the interaction (T_1 x T_2) was non-significant in both seasons ($P = 0.382$ and $P = 0.331$, respectively). The largest reduction in germination occurred in T_3. The reduction was greater in 2012–13 (−16%) than in 2011–12 (−7%) (Table 2). When averaged data for the two seasons were analysed, T_1 did not reduce germination but T_2 did so, and T_3 had the lowest germination (Table 2).

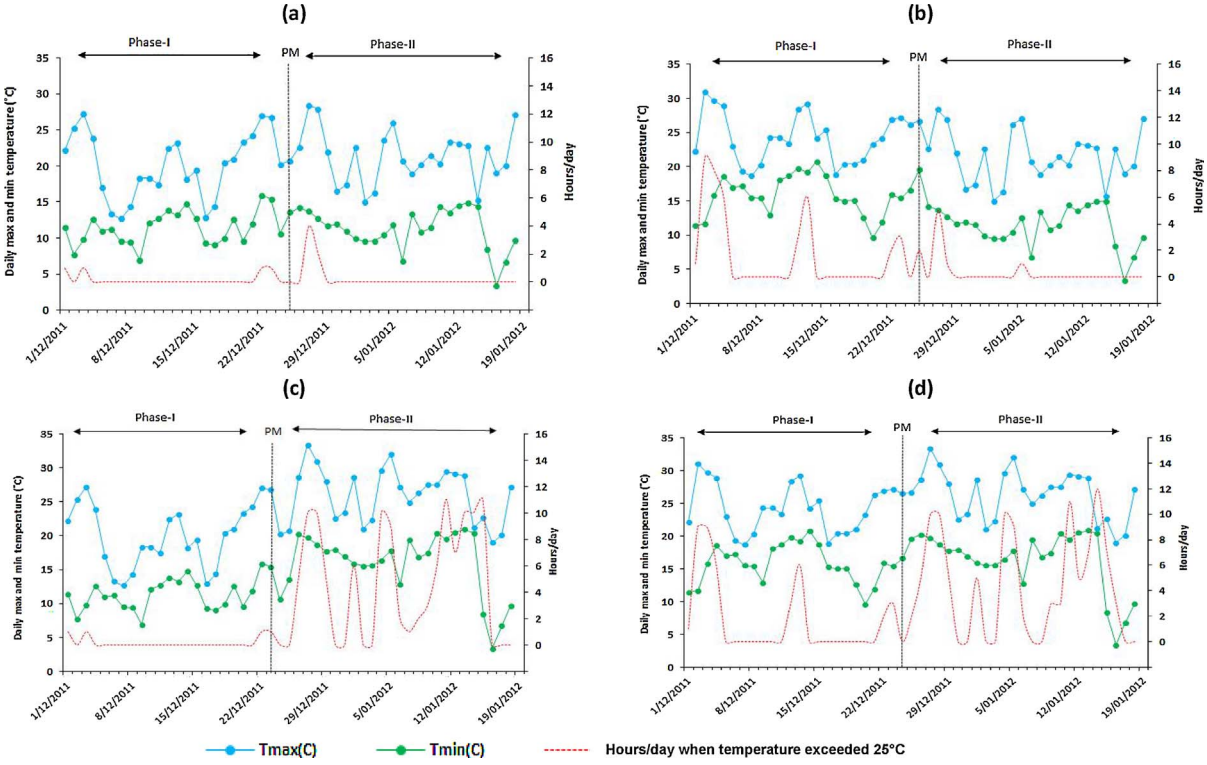

Fig. 2. Daily maximum and minimum temperature and number of hours per day when temperature exceeded 25° C in the 2011–12 season. (a) Control plants, section of plots exposed to ambient field temperature (b) section of plots covered with plastic sheets for T_1 (c) section of plots covered with plastic sheets for T_2 (d) section of plots covered with plastic sheets for T_3.

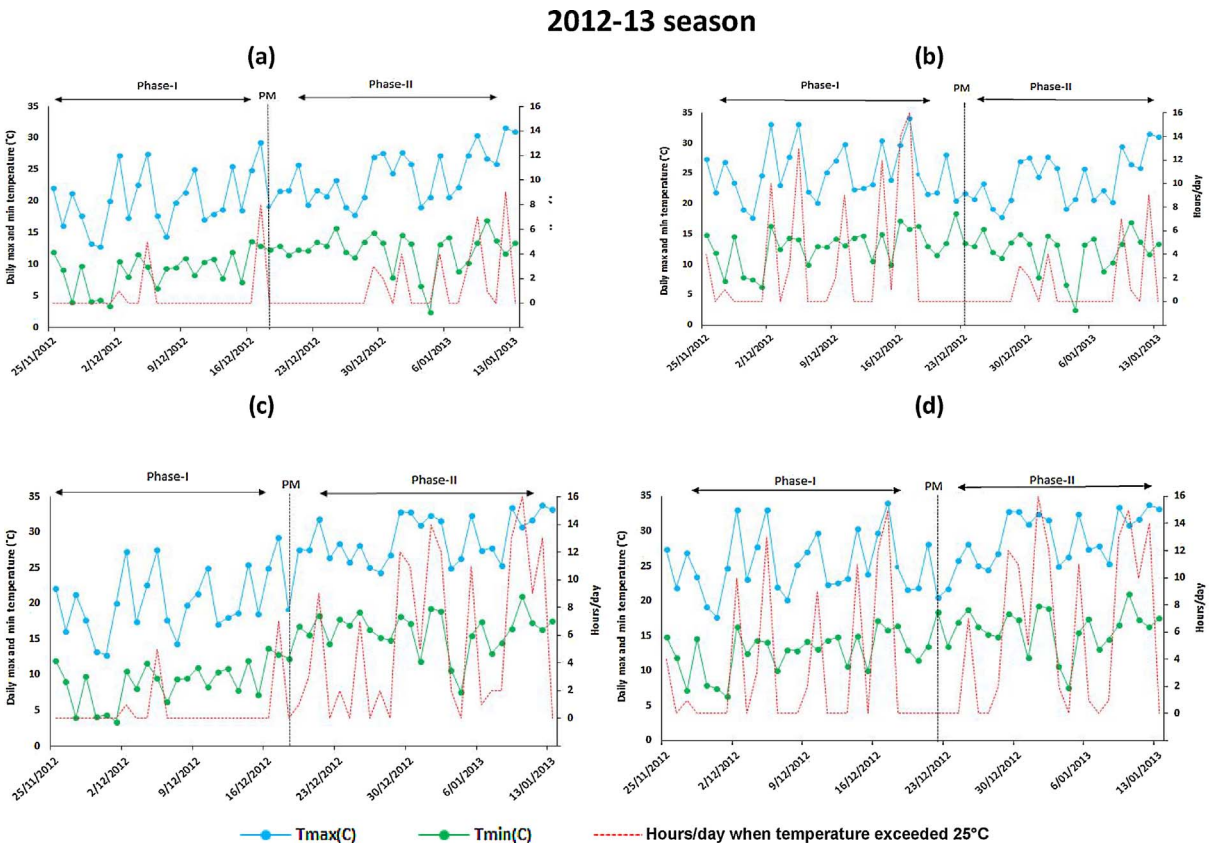

Fig. 3. Daily maximum and minimum temperature and number of hours per day when temperature exceeded 25 °C in the 2012-13 season. (a) Control plants, section of plots exposed to ambient field temperature (b) section of plots covered with plastic sheets for T_1 (c) section of plots covered with plastic sheets for T_2 (d) section of plots covered with plastic sheets for T_3.

Thousand seed weight was significantly ($P < 0.001$) reduced following the heat stress before (T_1) but not after (T_2) physiological maturity. The maximum reduction in thousand seed weight was recorded for heat stress T_3 in both seasons. However, there was no significant interaction between heat stress treatments before and after physiological maturity (T_1 X T_2) in either season (Table 2).

Germination of seeds harvested from control plants after accelerated ageing was ≈ 90% in both seasons. Treatments T_1, T_2 and T_3 all significantly reduced seed vigour as determined by the accelerated ageing test in both seasons (Table 3). The main effect of heat stress at T_1 and T_2 significantly reduced seed vigour in both seasons ($P < 0.001$). However, the negative effect of heat stress was larger for treatment T_3 in 2012–13 (32%) than in 2011–12 (15%) (Table 3). For both seasons, the seeds harvested from control plants had the lowest conductivity. All three heat stress treatments significantly increased conductivity. Heat stress before physiological maturity (T_1) significantly ($P < 0.001$) increased conductivity in both seasons relative to the control, as did heat stress after physiological maturity (T_2). The largest increase in conductivity was found for heat stress T_3. There was no significant interaction between T_1 and T_2 in either season, ($P = 0.185$ and $P = 0.451$, respectively) (Table 3).

Table 1
Effect of covering forage rape plots with plastic sheeting to increase temperature during seed development on number of hours > 25 °C and hourly thermal time (HTT; Tbase = 25°C). SMC = seed moisture content, RH = relative humidity.

Treatment	Growing season	T_1 (before physiological maturity) SMC (≈ 80-50%)				T_2 (after physiological maturity) SMC (50-14%)				SMC (≈80-14%)		
		Period	No. of hours	HTT	R.H	Period	No. of hours	HTT	R.H	No. of hours	HTT	R.H
			(> 25 °C)	(°C h)	(%)		25 °C	(°C h)	%	(> 25 °C)	(°C h)	(%)
T_o	2011-12	2 Dec-23 Dec	3	6.7	81	24 Dec-10 Jan	6	8.9	78	9	15.6	79
	2012-13	25 Nov-18 Dec	14	28.1	75	19 Dec-8 Jan	16	23.4	72	30	51.5	74
T_1	2011-12	2 Dec-26 Dec	40	101.3	86	27 Dec-13 Jan	7	9.7	79	47	111.0	82
	2012-13	25 Nov-22 Dec	84	255.5	78	23 Dec-11 Jan	18	27.5	69	102	283.0	74
T_2	2011-12	2 Dec- 23 Dec	3	6.7	81	24 Dec-15 Jan	118	333.4	82	121	340.1	81
	2012-13	25 Nov-18 Dec	14	28.1	75	19 Dec-13 Jan	159	577.6	71	173	605.7	73
T_3	2011-12	2 Dec-25 Dec	38	6.9	87	26 Dec-16 Jan	111	328.3	81	149	425.2	84
	2012-13	25 Nov-22 Dec	81	247.5	80	23 Dec-14 Jan	147	535.7	70	228	783.2	76

T_0 = Control plants, section of plots not covered with plastic sheets; T_1 = Plants covered with plastic sheets between ≈80% SMC and physiological maturity (50% SMC); T_2 = Plants covered with plastic sheets between 50% SMC and seed harvest (14% SMC); T_3 = Plants covered with plastic sheets between ≈80% SMC and seed harvest (14% SMC).

Table 2
Effect of elevated temperature during seed development on forage rape seed germination and thousand seed weight (TSW) in the 2011–12 and 2012-13 seasons. HS = heat stress, T_1 = heat stress before seed physiological maturity, T_2 = heat stress after seed physiological maturity.

Treatments	Germination (%)			TSW (g)		
	2011 – 12	2012 – 13	Mean	2011 – 12	2012 – 13	Mean
Main effect of T_1						
Nil (no stress)	90.7	87.0	88.9	2.88	2.97	2.92
HS at T_1	88.9	80.8	84.9	2.60	2.60	2.60
LSD (5%)	1.3	1.3	6.6	0.13	0.07	0.10
Significance of difference	*	***	*ns*	***	***	**
Main effect of T_2						
Nil (no stress)	92.1	89.0	90.6	2.80	2.81	2.81
HS at T_2	87.5	78.8	83.2	2.68	2.76	2.71
LSD (5%)	1.3	1.3	6.6	0.13	0.07	0.10
Significance of difference	***	***	*	*ns*	*ns*	*ns*
Treatment means						
Control = T_0	92.8	92.4	92.6	2.96	3.00	2.98
HS at T_1	91.5	85.7	88.6	2.64	2.63	2.63
HS at T_2	88.7	81.6	85.2	2.80	2.93	2.86
HS at $T_1 + T_2 = T_3$	86.3	76.0	81.2	2.55	2.58	2.57
LSD (5%)	1.9	1.8	9.3	0.18	0.10	0.14
Interaction effect (T_1 x T_2)	−1.1	1.2	0.0	0.07	0.02	0.06
L.S. interaction (5%)	2.7	2.5	13.1	0.25	0.14	0.20
Significance of interaction	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

ns = Non significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$; *** = Significant at $P < 0.001$; Main effect of T_1; Nil (no stress) = $[T_0 + T_2]/2$; $T_1 = [T_1 + T_3]/2$; Main effect of T_2; Nil (no stress) = $[T_0 + T_1]/2$; $T_2 = [T_2 + T_3]/2$; Interaction Effect (T_1xT_2) = $[T_3$-$T_1]$-$[T_2$–$T_0]$; LSD (Main Effect) = LSD (Treatment means)/√2; LS. Interaction = LSD (Treatment means) x√2.

3.3. Relationship between hourly thermal time and seed quality

Analysis of covariance showed that differences in germination, germination after accelerated ageing, conductivity and thousand seed weight between the two seasons, after adjustment for hourly thermal time, were not significant (Fig. 4). Hourly thermal time was significantly correlated with germination ($P < 0.001$), germination after accelerated ageing ($P < 0.01$) and conductivity ($P < 0.01$), but not with thousand seed weight ($P = 0.06$). As hourly thermal time increased seed germination and vigour were reduced (Fig. 4).

3.4. Relationship between the number of hours when temperature was > 25 °C and seed quality

As found in 3.3, the differences in seed quality attributes between the two seasons, after adjustment for 'number of hours that temperature was > 25 °C', were not significant (Fig. 4). The 'number of hours that temperature was > 25 °C' was significantly correlated with germination ($P < 0.001$), germination after accelerated ageing ($P < 0.01$) and conductivity ($P < 0.001$), but not with thousand seed weight ($P = 0.114$). As hours > 25 °C increased, seed germination and vigour

Table 3
Effect of elevated temperature during seed development on forage rape seed vigour in the 2011–12 and 2012-13 seasons. HS = heat stress, T_1 = heat stress before seed physiological maturity, T_2 = heat stress after seed physiological maturity.

Treatments	Accelerated ageing vigour test germination (%)			Conductivity (μS cm^{-1} g^{-1})		
	2011 – 12	2012 – 13	Mean			Mean
Main effect of T_1						
Nil (no stress)	85.4	82.4	83.9	54.1	62.5	58.3
HS at T_1	79.6	69.0	74.3	74.9	83.0	78.9
LSD (5%)	2.1	5.6	12.1	5.9	4.5	3.4
Significance of difference	***	***	*ns*	***	***	***
Main effect of T_2						
Nil (no stress)	87.2	85.2	86.2	54.1	60.3	56.9
HS at T_2	77.8	66.2	72.0	75.9	85.2	80.3
LSD (5%)	2.1	5.6	12.1	5.9	4.5	3.4
Significance of difference	***	***	*	***	***	***
Treatment means						
Control = T_0	90.2	88.8	89.5	41.3	49.3	45.3
HS at T_1	84.2	81.6	82.9	65.9	71.4	68.6
HS at T_2	80.6	75.9	78.2	66.9	75.7	71.3
HS at $T_1 + T_2 = T_3$	75.1	56.5	65.8	84.0	94.6	89.3
LSD (5%)	2.9	7.9	17.0	8.4	6.3	4.8
Interaction effect ($T_1 \times T_2$)	0.5	−12.2	−4.8	−7.5	−3.12	−5.3
L.S. interaction (5%)	4.1	11.2	24.0	11.8	8.9	6.8
Significance of interaction	*ns*	*	*ns*	*ns*	*ns*	*ns*

ns = Non significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$; *** = Significant at $P < 0.001$; -Main effect of T_1; Nil (no stress) = $[T_0 + T_1]/2$; $T_1 = [T_1 + T_3]/2$; Main effect of T_2; Nil (no stress) = $[T_0 + T_1]/2$; $T_2 = [T_2 + T_3]/2$; Interaction Effect (T_1xT_2) = $[T_3$-$T_1]$-$[T_2$–$T_0]$; LSD (Main Effect) = LSD (Treatment means)/√2; LS. Interaction = LSD (Treatment means) x√2.

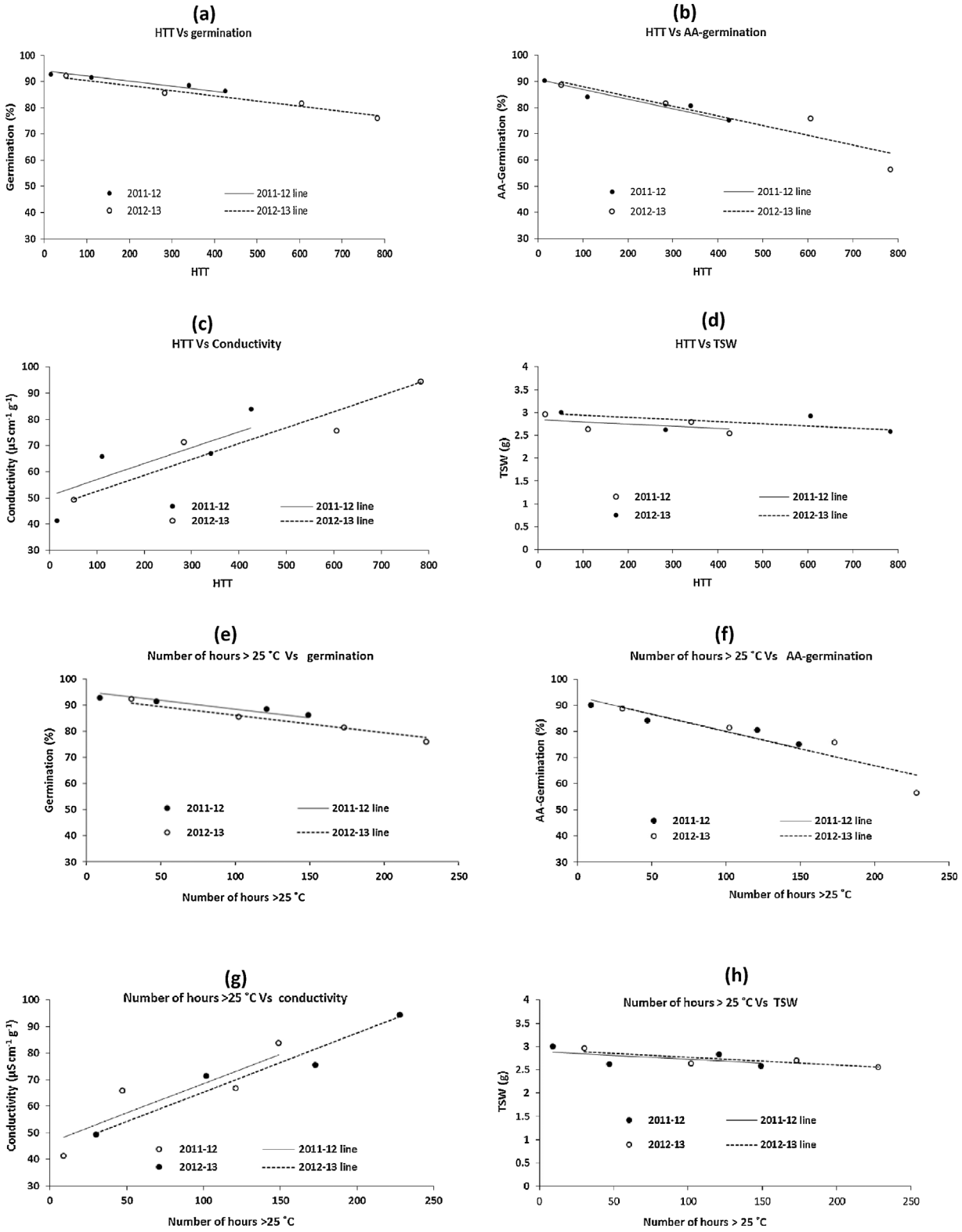

Fig. 4. Correlation within seasons (pooled between seasons using ANCOVA) between y − variables (a) seed germination percentage, (b) accelerated ageing germination percentage, (c) electrical conductivity and (d) thousand seed weight, and x – variable hourly thermal time, and between these same y – variables and x – variable 'number of hours when temperature exceeded 25 ˚C' in (e), (f), (g), and (h), respectively. The differences between the two seasons in the vertical (y) elevations of the fitted parallel lines were not significant in all of (a) to (h). The common slope of the parallel lines was (a) −0.0193, (b) −0.0373, (c) 0.061, (d) −0.00047, (e) −0.067, (f) −0.131, (g) 0.222 and (h) −0.000169. The significance of the common slope was (a) $P < 0.001$, (b) $P < 0.01$, (c) $P < 0.01$, (d) not significant ($P = 0.06$), (e) $P < 0.001$, (f) $P < 0.01$, (g) $P < 0.01$ and (h) not significant ($P = 0.112$). The latter P values can also be interpreted as the 'significance of the covariate' and the 'significance of the pooled correlation'.

were reduced (Fig. 4).

4. Discussion

In the field experiments, ambient air temperature data were acquired using HOBO data loggers, which are not World Meteorological Organisation level sensors. They may therefore have provided data which either over or under-represented air temperature. This was checked by comparing temperature data obtained from the Lincoln Broadfield Electronic Weather Station situated less than one kilometre from the trial site (Rashid et al., 2017b). For both seasons the two sets of temperature data differed by between 0.5–1.5 °C for mean maximum temperature and by between 1.5–2.4 °C for mean minimum temperature. In both seasons the higher temperature means were recorded from the Broadfield Station, suggesting that any errors resulting from the accuracy of the HOBO temperature data were not impacting on the heat stress responses recorded for seed quality.

In these field experiments, ambient air temperature was artificially increased by covering sections of the plots with plastic sheeting in an attempt to mimic possible effects of global warming on seed quality. The time between seeds reaching 80% and 14% seed moisture content was 42 days in 2011/12 (2nd December to 13 January) and 37 days in 2012/13 (13 December to 19 January). In control plots during these time periods, plants were exposed to temperatures exceeding 25 °C for 9 h in the first season and for 30 h in the second season, with the corresponding hourly thermal time being 15.6 °C and 51.5 °C respectively. Covering the plots resulted in hourly thermal time values ranging from 110 °C to 425 °C in 2011/12, and from 283 °C to 783 °C in 2012/13. It could be argued that because these hourly thermal time levels were so much greater than for the control, they were unrealistic and would be unlikely to be reached in the Canterbury environment. However, Shinohara et al. (2006a) recorded hourly thermal time (Tb = 25 °C) in a field trial sited less than one kilometre from our forage rape trials and reported that for the period 29 December to 13 January (15 days), hourly thermal time was 326 °C, and during this time plants received 40 h of temperatures exceeding 30 °C. This hourly thermal time value from the 2003/2004 seson is higher than that created by T_1 in both 2011/12 and 2012/13, and equivalent to that created by T_2 in the former season. It is therefore possible that the predicted increase in heat stress events (NIWA, 2012) could produce hourly thermal time values exceeding 400 °C h in Canterbury summers.

Seeds are known to be sensitive to heat stress both before and after they reach physiological maturity (Hampton et al., 2013; Prasad et al., 2015). Rashid et al. (2017a) demonstrated that a short period of heat stress of 4 days at 30 °C day/25 °C night (240 °C h) during forage rape seed development (80% SMC) reduced seed germination, seed vigour and seed mass, and that the same heat stress at physiological maturity (50% SMC) reduced seed germination and vigour, but not seed mass. A similar response to the same length and timings of heat stress was reported by Shinohara et al. (2006b) for garden pea (*Pisum sativum* L.). In the present study, increasing hourly thermal time significantly reduced seed germination and vigour.

While heat stress did reduce germination, the loss in germination was not large (4% for T_1, 7% for T_2 and 11% for T_3 when data for the two seasons were averaged). As also reported by Rashid et al. (2017a), the heat stress did not kill seeds. These small reductions in germination were because of the production of abnormal seedlings (ISTA, 2016) mostly associated with root defects (Rashid et al., 2017a). Abnormal seedling production can result from mechanical injury to the embryo, plant pathogen attack, or physiological damage to the seed (Gillen et al., 2012). Heat stress induces physiological deterioration of seeds (Dornbos and McDonald, 1986; Toledo et al., 2011), possibly by disturbing the balance between reactive oxygen species (ROS) production and ROS scavenging enzymes, allowing uncontrolled accumulation of H_2O_2 and reduced energy supply through damage to the mitochondria, which can result in the production of abnormal seedlings (Bailly et al., 2008).

During the accelerated ageing vigour test, heat stressed seeds also germinated physiologically, but an increased percentage of the seedlings so produced were abnormal. This loss of seed vigour in response to heat stress as assessed by the accelerated ageing test was confirmed by the conductivity tests results. Rashid et al. (2017a) reported a significant negative relationship ($r = -0.981$, $P < 0.05$) between the conductivity and accelerated ageing germination of forage rape seed lots. Heat stress is known to disrupt normal structure and function of cell organelles including mitochondrial activity and cell membranes, reducing respiratory activity (Grass and Burris, 1995) and membrane function (Fath et al., 2002; Ren et al., 2009), thereby reducing seed vigour (McDonald, 1999). Rashid (2016) confirmed that this reduction in seed vigour was directly related to H_2O_2 accummulation, lipid peroxidation, reduced antioxidant enzyme activity and lowered ATP levels.

Heat stress before physiological maturity is known to reduce seed mass (Morrison and Stewart, 2002; Gan et al., 2004; Yu et al., 2014; Rashid et al., 2017a). As noted by Rashid et al. (2017a), it is likely that heat stress during seed development reduced assimilate supply to the seeds, negatively affecting seed mass (Morrison and Stewart, 2002). Heat stress after physiological maturity did not affect seed mass because by this time the seeds had completed their development and achieved their maximum dry weight.

New Zealand-produced forage rape seed lots usually have a germination of > 90%, but can vary considerably in seed vigour (Rashid et al., 2017a). Seed lot vigour status affects their ability to emerge in the field once sown, particularly in stress environments (Powell, 1988; Hampton, 2000; Finch-Savage and Bassel, 2016). While there are as yet no industry standards for seed vigour, Egli and TeKrony (1995, 1996) suggested that for soybean (*Glycine max* Merril.), an accelerated ageing vigour test germination of 80% was suitable as a minimum standard for sowing seed in a wide range of environments. From our forage rape data, approximately 300 °C h (T_b = 25 °C) or 100 h > 25 °C were required to reduce accelerated ageing-germination to 80%.

In the present study, most of the times when temperature was > 25 °C occurred from the first week of December until the middle of January in both seasons. One strategy suggested to reduce the seed quality loss caused by heat stress was to change the sowing date to avoid heat stress during seed development and maturation (Hampton et al., 2013). However, this may be difficult to achieve. Rashid et al. (2017b) sowed forage rape seed crops in March and April (sowing three weeks apart) at the same site, and found only an eight day difference in the time to reach physiological maturity and harvest maturity. An added complication is that seed vigour can be lost following heat stress both before, at and after physiological maturity. For forage rape the time between the start of seed development and harvest maturity is around 40 days in the Canterbury environment. Trying to avoid any heat stress during this time will be difficult. A second option may be to move seed production further south in New Zealand (Hampton et al., 2013), but this is yet to be explored. It is more likely however that breeding for heat stress tolerance or escape (Yu et al., 2014; Prasad et al., 2017) will better equip growers to cope with an increasing frequency of heat stress events.

Acknowledgements

M. Rashid thanks the Higher Education Commission, Pakistan and Lasbella University, Pakistan for the provision of a Faculty Development Scholarship and the Bio-Protection Research Centre for providing a CoRE research scholarship during his PhD studies. This work was supported by funding from the New Zealand Ministry of Business, Innovation and Employment (LINX0803 Advanced Seed Production Systems).

References

Aksouh-Harradj, N.M., Campbell, L.C., Mailer, R.J., 2006. Canola response to high and moderately high temperature stresses during seed maturation. Can. J. Plant Sci. 86, 967–980.

Bailly, C., El-Maarouf-Bouteau, H., Corbineau, F., 2008. From intracellular signalling networks to cell death: the dual role of reactive oxygen species in seed physiology. C. R. Biol. 331, 806–814.

Dornbos, D., McDonald, M., 1986. Mass and composition of developing soybean seeds at five reproductive growth stages. Crop Sci. 26, 624–630. http://dx.doi.org/10.2135/cropsci1986.0011183x002600030042x.

Egli, D., TeKrony, D., 1995. Soybean seed germination, vigor and field emergence. Seed Sci. Technol. 23, 595–607.

Egli, D., TeKrony, D., 1996. Seedbed conditions and prediction of field emergence of soybean. J. Prod. Agric. 9, 365–370.

Fath, A., Bethke, P., Beligni, V., Jones, R., 2002. Active oxygen and cell death in cereal aleurone cells. J. Exp. Bot. 53, 1273–1282.

Finch-Savage, W.E., Bassel, G.W., 2016. Seed vigour and crop establishment: extending performance beyond adaptation. J. Exp. Bot. 67, 567–591. http://dx.doi.org/10.1093/jxb/erv490.

Gan, Y., Angadi, S., Cutforth, H., Potts, D., Angadi, V., McDonald, C., 2004. Canola and mustard response to short periods of temperature and water stress at different developmental stages. Can. J. Plant Sci. 84, 697–704.

Gillen, A.M., Smith, J.R., Mengistu, A., Bellaloui, N., 2012. Effects of maturity and *Phomopsis longicolla* on germination and vigor of soybean seed of near-isogenic lines. Crop Sci. 52, 2757–2766. http://dx.doi.org/10.2135/cropsci2011.10.0566.

Grass, L., Burris, J., 1995. Effect of heat stress during seed development and maturation on wheat (*Triticum durum*) seed quality. II. Mitochondrial respiration and nucleotide pools during early germination. Can. J. Plant Sci. 75, 831–839.

Hampton, J.G., Rolston, M.P., Pyke, N., Green, W., 2012. Ensuring the long term viability of the New Zealand seed industry. Agron. N.Z. 42, 129–140. http://dx.doi.org/10.1017/S0021859612000263002l-8596.

Hampton, J.G., Boelt, B., Rolston, M.P., Chastain, T., 2013. Effects of elevated CO_2 and temperature on seed quality. J. Agric. Sci. 151, 154–162.

Hampton, J.G., 2000. Producing quality seed: the problem of seed vigor. In: McManus, M.T., Outred, H.A. (Eds.), Current Research on Seeds in New Zealand. Agron. Soc. N.Z. Special Publication 12, Palmerson North, New Zealand, pp. 53–67.

ISTA, 2016. International Rules for Seed Testing. International Seed Testing Association, Bassersdorf, Switzerland.

McDonald, M.B., 1999. Seed deterioration: physiology, repair and assessment. Seed Sci. Technol. 27, 177–237.

Morrison, M.J., Stewart, D.W., 2002. Heat stress during flowering in summer brassica. Crop Sci. 42, 797–803.

NIWA, 2012. Drought in a Changing Climate. A Summary from Scenarios of Regional Drought Under Climate Change. (Retrieved from www.niwa.co.nz).

Powell, A.A., 1988. Seed vigor and field establishment. Adv. Res. Technol. Seeds 11, 29–61.

Prasad, P., Staggenborg, S., Ristic, Z., 2008. Impacts of drought and/or heat stress on physiological, developmental, growth and yield processes of crop plants. In: Ahuja, L.H., Saseendran, S.A. (Eds.), Response of Crops to Limited Water: Understanding and Modelling Water Stress Effects on Plant Growth Processes. ASA-CSSA, Madison, WI USA, pp. 301–355.

Prasad, P.V., Djanaguiraman, M., Perumal, R., Ciampitti, I.A., 2015. Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Front. Pl. Sci. 6, 1–11. http://dx.doi.org/10.3389/fpls.2015.00820.

Prasad, P.V.V., Bheemanahalli, R., Krishna Jagadish, S.V., 2017. Field crops and the fear of heat stress –opportunities: challenges and future directions. Field Crops Res. 200, 114–121.

Rashid, M., Hampton, J.G., Rolston, M.P., Khan, K.M., Saville, D.J., 2017a. Heat stress during seed development affects forage brassica (*Brassica napus* L.) seed quality. J. Agron. Crop Sci (submitted).

Rashid, M., Hampton, J.G., Trethewey, J.A.K., Rolston, M.P., 2017b. Effect of sowing date on forage rape seed quality. Agron. N.Z. 47, 55–64.

Rashid, M., 2016. Effect of the Environment During Seed Development on Brassica Seed Quality PhD Unpublished. Lincoln University, Canterbury, New Zealand.

Ren, C., Bilyeu, K.D., Beuselinck, P., 2009. Composition, vigor and proteome of mature soybean seeds developed under high temperature. Crop Sci. 49, 1010–1022.

Shinohara, T., Hampton, J.G., Hill, M.J., 2006a. Effects of the field environment before and after seed physiological maturity on hollow heart occurrence in garden pea (*Pisum sativum* L.). N.Z.J. Crop Hort. Sci. 34, 247–256. http://dx.doi.org/10.1080/01140671.2006.9514414.

Shinohara, T., Hampton, J.G., Hill, M.J., 2006b. Location of deterioration within garden pea (*Pisum sativum*) cotyledons is associated with the timing of exposure to high temperature. N.Z.J. Crop Hort. Sci. 34, 299–309. http://dx.doi.org/10.1080/01140671.2006.9514420.

Singh, R.P., Prasad, P.V., Reddy, K.R., 2013. Impacts of changing climate variability on seed production and seed industry. Adv. Agron. 129, 117–180.

Spears, J., TeKrony, D., Egli, D., 1997. Temperature during seed filling and soybean seed germination and vigor. Seed Sci. Technol. 25, 233–244.

Toledo, M.Z., Teixeira, R.N., Ferrari, T.B., Ferreira, G., Cavariani, C., Cataneo, A.C., 2011. Physiological quality and enzymatic activity of crambe seeds after the accelerated ageing test. Acta Scient. Agron. 33, 687–694.

Trethewey, J.T., 2012. Crop management strategies to improve forage rape seed yield. Agron. N.Z. 42, 111–117.

Yu, E., Fan, C., Yang, Q., Li, X., Wan, B., Zhou, Y., 2014. Identification of heat response genes in *Brassica napus* siliques at the seed-filling stage through transcriptional profiling. PLoS One 9, 7. http://dx.doi.org/10.1371/journal.pone.0101914.